

## Amendments to the Claims

1-19 (Canceled)

20. (Currently amended) A ~~plasmid having a nucleic acid molecule~~sequence region comprising an open reading frame encoding a cleavable single-chain polypeptide, said open reading frame comprising:

a) ~~a first nucleotide sequence region comprising encoding at least a portion of a clostridial neurotoxin heavy chain binding element able to preferentially interact with a target cell surface marker under physiological conditions;~~

~~i) a first portion encoding a first amino acid sequence region comprising a binding element able to specifically bind a target cell surface marker under physiological conditions; and~~

b) ~~a second nucleotide sequence encoding at least a portion of a clostridial neurotoxin heavy chain translocation element able to facilitate the transfer of said single-chain polypeptide across a vesicular membrane;~~

~~ii) a second portion encoding a second amino acid sequence region comprising a translocation element able to facilitate the transfer of a polypeptide across a vesicular membrane;~~

[[b]]c) ~~a second~~third nucleotide sequence region encoding a ~~third amino acid sequence region comprising at least a portion of a therapeutic element~~ peptide having biological activity when released into the cytoplasm of the target cell, and

d) ~~a fourth nucleotide sequence encoding a peptide comprising a non-native Clostridial neurotoxin protease cleavage site;~~

wherein said fourth nucleotide sequence intervenes between said second sequence and said third nucleotide sequence.

~~wherein said first and second nucleotide sequence regions are separated by a third nucleotide sequence region encoding a fourth amino acid sequence comprising a protease cleavage site which is cleaved when exposed to a protease, provided said third amino acid sequence region is not cleaved by a human protease or a protease normally expressed by a cell expressing said single-chain polypeptide, and wherein said single-chain polypeptide is expressed by said plasmid within a suitable host cell.~~

21. (Currently amended) The ~~plasmid~~molecule of claim [[20]]20, wherein said ~~first or second nucleotide sequence region~~open reading frame further comprises ~~a fifth nucleotide sequence encoding~~ encodes an amino acid sequence region ~~a peptide comprising a target-binding portion of a binding tag.~~

22. (Currently amended) The ~~plasmid~~molecule of claim [[21]]21, wherein said ~~binding tag comprises a target-binding portion of a polypeptide selected from the group consisting~~

~~efcomprises a His<sub>6</sub>, a monoclonal antibodiesantibody, a maltose binding protein, a glutathione-S-transferase, a protein A, and/or a calmodulin binding protein.~~

23. (Currently amended) The plasmidmolecule of claim ~~[[20]]~~20, wherein said ~~first nucleotide sequence region encodes at least a portion of~~binding element is a *Clostridium botulinum* neurotoxin heavy chain~~a clostridial neurotoxin heavy chain~~.
24. (Currently amended) The plasmidmolecule of claim ~~[[23]]~~20, wherein said ~~first nucleotide sequence region encodes at least a portion of~~translocation element is a *Clostridium botulinum* neurotoxin heavy chain.
25. (Currently amended) The plasmidmolecule of claim ~~[[23]]~~20, wherein said ~~first nucleotide sequence region encodes at least a portion of~~translocation element is a *Clostridium tetani* neurotoxin heavy chain.
26. (Currently amended) The plasmidmolecule of ~~either of claim 20 or 23~~20, wherein said ~~second nucleotide sequence region encodes at least a portion of~~therapeutic element peptide comprises a clostridial neurotoxin light chain.
27. (Currently amended) The plasmidmolecule of claim ~~[[26]]~~26, wherein said ~~second nucleotide sequence region encodes at least a portion of~~clostridial neurotoxin light chain is a *Clostridium botulinum* neurotoxin light chain.
28. (Currently amended) The plasmidmolecule of claim ~~[[26]]~~26, wherein said ~~second nucleotide sequence region encodes at least a portion of~~clostridial neurotoxin light chain is a *Clostridium tetani* neurotoxin light chain.
- 29-31. (Canceled)
32. (Currently amended) A method of making a cleavable single-chain polypeptide ~~derived from a clostridial neurotoxin~~ comprising:
- a) inserting the plasmid of any one of claims ~~20-25 or 29-31~~20-28, 31 or 38 into a suitable host cell,
  - b) growing said host cell in culture, and
  - c) permitting or inducing the host cell to express the single chain polypeptide encoded by said plasmid.
33. (Currently amended) A method of purifying a ~~recombinant~~cleavable single chain polypeptide ~~derived from a clostridial neurotoxin~~ comprising:
- a) lysing a host cell expressing a single chain polypeptide from the plasmid of either of claim 21 or 22 to produce a cell lysate,
  - b) contacting said cell lysate with a target compound so as to form a specific binding complex capable of being immobilized comprising said binding tag and said target compound, and
  - c.) separating said binding complex from said cell lysate.
- 34-37. (Canceled)

38. (New) The molecule of claim 20, wherein said binding element is a *Clostridium tetani* neurotoxin heavy chain.
39. (New) The molecule of claim 20, wherein said protease cleavage site comprising SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 22 or SEQ ID NO: 23.
40. (New) A nucleic acid molecule comprising an open reading frame encoding a cleavable single-chain polypeptide, said open reading frame comprising:
- a) a first nucleotide sequence encoding at least a portion of a binding element peptide able to preferentially interact with a sensory afferent neuron cell surface marker under physiological conditions;
  - b) a second nucleotide sequence encoding at least a portion of a clostridial neurotoxin heavy chain translocation element able to facilitate the transfer of said single-chain polypeptide across a vesicular membrane;
  - c) a third nucleotide sequence encoding at least a portion of a clostridial neurotoxin light chain therapeutic element having biological activity when released into the cytoplasm of said target cell; and
  - d) a fourth nucleotide sequence encoding a peptide comprising a non-native Clostridial neurotoxin protease cleavage site;
- wherein said fourth nucleotide sequence intervenes between said second sequence and said third nucleotide sequence.
41. (New) The molecule of claim 40, wherein said open reading frame further comprises a fifth nucleotide sequence encoding a peptide comprising a target-binding portion of a binding tag.
42. (New) The molecule of claim 41, wherein said target-binding portion comprises a His<sub>6</sub>, a monoclonal antibody, a maltose binding protein, a glutathione-S-transferase, a protein A or a calmodulin binding protein.
43. (New) The molecule of claim 40, wherein said translocation element is a *Clostridium botulinum* neurotoxin heavy chain.
44. (New) The molecule of claim 40, wherein said translocation element is a *Clostridium tetani* neurotoxin heavy chain.
45. (New) The molecule of claim 40, wherein said therapeutic element is a *Clostridium botulinum* neurotoxin light chain.
46. (New) The molecule of claim 40, wherein said therapeutic element is a *Clostridium tetani* neurotoxin light chain.

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47. (New) The molecule of claim 40, wherein said protease cleavage site comprising SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 22 or SEQ ID NO: 23.